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Practitioner's Docket No.: 1372.17 -USF00A027

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Jaroszeski et al.)

Serial No.: 09/772,561)

Filing Date: 01/30/2001)

For: NONPENETRATING ELECTROPORATION)
DEVICE AND METHOD)

Examiner: Unk.

Art Unit: 3763

Faxed to Technology Center 3700 at (703) 872-9302
Box Fee Amendment
Hon. Commissioner for Patents
Washington, D.C. 20231

EXHIBIT A

(37 C.F.R. § 1.121)

Dear Sir:

Pursuant to 37 C.F.R. § 1.121 amendments are marked up to show all changes relative to the previous version of the above-mentioned application on this separate document.

IN THE SPECIFICATION:

Please amend the specification as follows, in accordance with 37 C.F.R. 1.121(b)(1)(iii):

Page 2, the paragraph starting at line 11:

The physical nature of electroporation makes it universally applicable. A variety of procedures utilize this type of treatment, which gives temporary access to the cytosol. These include the production [on] of monoclonal [proteins] antibodies, and genetic transformation. In addition, dyes and fluorescent molecules have been used to investigate the phenomenon of electroporation. A notable example of loading molecules into cells in vivo is electrochemotherapy. The procedure utilizes a drug combined with electric pulses as a means for loading tumor cells with an anticancer drug, and has been performed in a

number of animal models and in clinical trials by the present inventors. Also, plasmid DNA has been loaded into rat liver cells in vivo (Heller et al., FEBS Lett. 389, 225-28).

Page 2, the paragraph starting at line 21:

Protocols for the use of electroporation to load cells in vitro typically use a suspension of single cells or cells that are attached in a planar manner to a growth surface. In vivo electroporation is more complex because tissues are involved. Tissues are composed of individual cells that collectively make up a three-dimensional structure. In either case, the effects on the cell are the same. [FIGURE] FIG 1 illustrates details of the electroporation procedure. Electrodes and electrode arrays for delivering electrical waveforms for therapeutic benefit, including inducing electroporation, have been described by Bernard (WO 98/47562).

Page 4, the paragraph starting at line 8:

Electropermeabilization of tumor cell membranes has been reported (Rols et al., Nature Biotechnology 16, 173, 1998) using applied electric pulses from surface electrodes in contact with the skin. Proteins and DNA can be transferred into the cells by incorporating either the protein or DNA carrying a reporter gene. The efficiencies of transfer for the protein and DNA were, respectively, 20% and 4%.

Page 8, the paragraph starting at line 18:

FIG. 4 is a bottom plan view of [the embodiment of] the embodiment of FIG. 3.

Page 12, the paragraph starting at line 7:

Each electrode 20 is in circuit communication with a respective portion of the source 1 of electrical energy. In a preferred embodiment this source comprises a pulse generator such as is known in the art (e.g., a PA-2000 or PA-4000, both from Cyto Pulse sciences, Inc., Columbia, MD; a T820, BTX, Inc., San Diego, CA) and adapted to deliver pulses of a predetermined shape, voltage, duration, and separation. In particular, the source 1 should be adapted to deliver voltage to each electrode 20 for establishing a first, low-

level and a second, typically higher-level electromagnetic field in vivo between selected electrodes. Selective control of the application of electrical signals between the individual electrodes can be accomplished in different ways, e.g., via the PA-201 Programmable Pulse Switch in combination with the PA-4000 generator (both from Cyto Pulse Sciences, Inc., Columbia, MD), or it can be done manually, mechanically, or electrically. Based on the particular need of the application of the system, the electrical energy may include, but are not limited to, rectangular direct current pulses, exponentially decreasing DC pulses, alternating current, exponentially increasing DC pulses, bipolar DC pulses, DC biased DC waveforms, DC biased AC waveforms, pulsed alternating current, and radio frequency waves. The system may also be controlled by a computer system with the appropriate software designed to enable selective control of the signal generator as defined by the [needs of the target tissue] electrode, target tissue, and/or specific treatment.

Page 16, the paragraph starting at line 15:

Details of the electrodes 68-71 in this embodiment will be presented for electrode 68, with the understanding that the other electrodes 69-71 have a similar configuration. (In FIG. [10] 15, the electrode 69 is shown only partially to provide an inner view of electrode insulation 75.) Preferably the electrodes 68-71 comprise generally rectangular, elongated striplike members having insulation extending from the proximal end 84. This insulation electrically isolates each electrode member and can extend all the way from the proximal end 84 all the way to the distal end 80, leaving [only] enough electrode surface exposed to allow [proper] energy transfer from the electrode members to the target tissue T. This insulation may be a separate member, a surface coating applied to the actual electrode member, or other type of insulation as known to those of skill in the art. At the distal end 80, the electrode 68 comprises a distal portion 80 that is electrically exposed at least on the side 82 facing the other electrodes to permit delivering a pulse therefrom. In a preferred embodiment the facing side 82 is substantially planar, although this geometry may be altered to suit a desired target tissue T. For example, in FIG. 12 is illustrated an alternate embodiment 100, wherein the electrodes 104-107 are curved to facilitate tissue contact. In addition, this embodiment shows the addition of barbs 108 to the distal ends

80 of the electrodes that serve as gripping means with respect to the target tissue T. These barbs are not limited to use solely in this embodiment, it is understood that this feature may be incorporated into any of the electrodes disclosed herein.

Page 20, the paragraph starting at line 10:

Further examples of tissue-contacting portions of the electrode structures are shown in FIGS. 22 and 23A-F. In these embodiments, the tissue contact member is able to be noncontiguous (FIG. 22) as opposed to the member shown in FIG. 9. Alternative geometries for the contact members are also within the scope of the invention (FIGS. 23A-F), and these can be square, rectangular, elliptical, triangular, kidney-shaped, free-form, or any other shape configured to the needs of the system as defined by the target tissue. The various electrode members may also have alternate shapes and sizes as shown by items 140-142. The shapes of the various electrodes are exemplary only and are not intended to be limited, but any shape is useable as available to one of ordinary skill in the art. [In addition to the various shapes as shown, either single-conductivity electrode members or the multiple-polarity electrodes disclosed above may be used in these geometries.]

Page 21, the paragraph starting at line 18:

A second electrical potential is established between a pair of electrodes, which may or may not be the same poles on the multipolar electrode or pairs in the case of unitary polar members 20 and 21 as previously activated. The second potential is higher than the first electrical potential and is sufficient to cause electroporation in the target tissue T to enhance a movement of the desired molecule M into a cell. Exemplary field strengths and duration ranges include, but are not intended to be limited to, 1-10,000 V/cm in the nanosecond range to the millisecond range. In a particular embodiment the field strength range is 750-1500 V/cm over the microsecond to millisecond range. Either or both of the potentials can be delivered in a series of predetermined [sequence of] electrical pulses, each of which can comprise pulses delivered sequentially or simultaneously.

Page 23, the paragraph starting at line 10:

Another embodiment of a method of using one of the devices 10, 100, 110, or 120 comprises the step of introducing, such as by injection, although this is not intended as a limitation, a desired substance into the target tissue. The distal portions of the electrodes 68-72 are placed in contact with the tissue, and the sleeve 90, or the member 123 is moved to a position wherein the tissue area is [closely] encompassed by the electrodes 68-72, which ensures sufficient electrical contact therebetween. At least one, and preferably a plurality of, electrical pulses as desired are delivered to the electrodes 68-72 using the pulse generator 1, and thence to the tissue area for achieving electroporation and entrance of the desired substance into the target cells.

Page 24, the table beginning at line 21:

TABLE 1

Treatment Group	Number of Samples	Electrical Treatment	Mean Luciferase Expression
1	4	none	1,123,344
2	4	1500 V/cm 100 μ s	[-]1,735,343
3	4	1500 V/cm 100 μ s, followed by 17 V/cm 100 ms	[-]7,046,177
4	4	1500 V/cm 100 μ s, followed by 40 V/cm, 20 ms	17,692,651

IN THE CLAIMS:

Please cancel claims 5, 6, 8, 14-15, 17-20, 47 and 55-56. Please amend claims 1-3, 7, 12-13, 16, 20, 25-28, 33, 38, 40-41, 43-44, 46, 48-52, and 57-58 in accordance with 37 C.F.R. § 1.121(c)(1)(ii), i.e., bracketed terms are deleted from the claims and underscored terms are added thereto.

1. (Amended) A device for manipulating a molecule *in vivo* relative to a target tissue comprising a support and at least one electrode member extending away from and affixed to for defining the support, the at least one electrode member having [a plurality of] at least one conductive [portions and a nonconductive] portion, wherein:

the sum of electrode members and conductive portions equals at least three;

the conductive portions are [positioned in spaced-apart relation from each other] separated by nonconductive portions, each conductive portion being in circuit communication with a respective portion of a source of electrical energy;

the conductive portions are configured to establish a first electromagnetic field between selected conductive portions sufficient to manipulate a molecule relative to a target tissue and a second electromagnetic field sufficient to cause transient permeability of a cell membrane within the target tissue; and

at least two of the conductive portions are locatable against a selected portion of the target tissue.

2. (Amended) The device recited in Claim 1, wherein the conductive portions and the nonconductive portions are located on [a single support] the same electrode member.

3. (Amended) The device recited in Claim 1, wherein the conductive portions and nonconductive portions are located on separate [support] electrode members.

7. (Amended) The device recited in Claim 1, wherein the support comprises a generally cylindrical post having a portal therethrough from a top end to a bottom end and the device further comprises:

a disc affixed to the post bottom end, the disc having a bottom surface having an outer downwardly depending annulus comprising alternating [sectors of conductive areas and

nonconductive areas] conductive portions separated by nonconductive portions, the electrode member comprising the annulus and the conductive portions [comprising the conductive sectors] serving as electrodes; and

a lead in circuit communication with each conductive [area] portion extending from the disc through the post portal to the top end thereof.

12. (Amended) The device recited in Claim 7, wherein the disc comprises a [portion] section having sufficient transparency to permit visualization of the [target tissue selected portion] selected target tissue therethrough.

13. (Amended) The device recited in Claim 1, further comprising means for delivering a preselected pattern of signals to selected [pairs of the] conductive portions to effect a desired molecular result.

16. (Amended) The device recited in Claim 1, the electrode member further comprising a downwardly depending post affixed adjacent a bottom end of the support, the post having at least one conductive [area] portion on a surface thereof.

21. (Amended) The device recited in Claim 20, wherein the [post] electrode member comprises a plurality of downwardly depending posts, each post axially movable between a first position and a second position lower than the first position and biased to the second position, for achieving contact between each post and a target tissue surface.

25. (Amended) The device recited in Claim 21, wherein each post has a pointed conductive bottom tip, the tips disposed at a radially inwardly facing angle to each other, each post inwardly movable between a first position and a second position wherein the tips are closer together than in the first position, the second position for [gripping tissue] achieving contact with the target tissue between the tips.

26. (Amended) The device recited in Claim 1, further comprising a pair of [electrode-bearing] electrode members movably affixed to the support in separation-adjustable fashion, each [electrode-bearing] electrode member comprising means for affixing at least one [electrode] conductive portion thereto, said conductive portions serving as electrodes.

27. (Amended) The device recited in Claim 26, wherein each [electrode-bearing] electrode member comprises an insulating plate, and wherein [the electrode members comprise] a plurality of electrodes affixed to [an inward-facing] a surface of each plate, the plates configured to [grip] contact at least a portion of the target tissue therebetween.

28. (Amended) The device recited in Claim 1, further comprising means for establishing at least [one pair of opposite-polarity] two substantially different voltages approximately simultaneously on [a respective pair] two or more conductive portions.

33. (Amended) The device recited in Claim 1, further comprising means [for facilitating attachment of the electrode member to the target tissue] to facilitate contact between the electrode member and the target tissue.

38. (Amended) A method for achieving a desired distribution and delivery of one or more [a] molecules from an initial location into a target tissue, the method comprising the steps of:

placing at least one [electrode-bearing] electrode member [containing areas of conductivity capable of having reverse polarities,] comprising at least one conductive portion, wherein at least two conductive portions are generally adjacent, but in nonpenetrating fashion to, a surface adjacent a target tissue, each [electrode] conductive portion in circuit communication with a respective portion of a source of electrical energy;

establishing a first electrical potential between [a pair of the areas of conductivity] at least two conductive portions sufficient to cause electromigration of the desired molecule from the initial location toward the target tissue; and

establishing a second electrical potential between [a pair of the areas of conductivity] at least two conductive portions [higher than the first electrical potential] sufficient to cause electroporation in the target tissue for enhancing a movement of the desired molecule into a cell thereof.

40. (Amended) The method recited in Claim 38, further comprising the step of establishing a third electrical potential between [a pair of areas of conductivity] at least two conductive portions sufficient to cause electromigration of the desired molecule from a location adjacent the target tissue through a pore in a cell membrane of the target tissue into an interior thereof.

41. (Amended) The method recited in Claim 40, wherein the establishing step[s] comprises establishing a series of [first, second and] third electrical potentials in a predetermined sequence of pulses.

43. (Amended) The method recited in Claim 38, wherein the electromigration is effected to cause the molecule to [be delivered beneath] penetrate a skin layer.

44. (Amended) A method for delivering a bioactive molecule from an initial location to a target tissue, the method comprising the steps of:

placing at least one electrode member having [areas of conductivity of opposite polarities] conductive portions, wherein at least two conductive portions are against a surface generally adjacent, but in nonpenetrating fashion to, a target tissue, each [member bearing

sections of reverse polarity, each electrode member] conductive portion serving as an electrode and being in circuit communication with a respective portion of a source of electrical energy;

activating [a pair of the areas of opposite polarity] at least two electrodes to achieve an electromigration of the bioactive molecule from the initial location to a location adjacent the target tissue; and

activating [a pair of the areas of conductivity] at least two electrodes to achieve electroporation of a cell membrane within the target tissue sufficient to permit entry of the biological molecule into the cell interior.

46. (Amended) A method for bringing [two molecules] a first and a second molecule from two respective initial locations into apposition at a desired target tissue site for permitting a reaction therebetween, the method comprising the steps of:

placing [an electrode member containing at least two areas of conductivity thereon against a surface adjacent a desired target tissue site;] at least one electrode member having conductive portions, wherein at least two conductive portions are against a surface generally adjacent, but in nonpenetrating fashion to, a target tissue, each conductive portion serving as an electrode and being in circuit communication with a respective portion of a source of electrical energy;

activating the [areas of conductivity] conductive portions to cause an electromigration of the first [and second] molecule to a third area adjacent the target tissue site;

activating the conductive portions to cause an electromigration of the second molecule to a third area adjacent the target tissue site; and

permitting the first and the second molecule to react at the third area.

48. (Amended) The method recited in Claim 46, wherein the electromigration of the first molecule is effected to cause the first [and the second] molecule to penetrate a skin layer.

49. (Amended) The method recited in Claim[s] 46, wherein the activation steps cause[s] the first and the second molecule to be delivered to [an internal compartment or cytosol] the cytosol of cells comprising the target tissue.

50. (Amended) The method recited in Claims [46] and 77, wherein the penetration step is effected through a biological tissue other than skin.

51. (Amended) The method recited in Claim 46, [wherein the activating step is sufficient to cause electromigration but is insufficient to cause electroporation] further comprising the step, prior to the activating step, of activating two conductive portions to cause electroporation in the target tissue.

52. (Amended) The method recited in Claim 46, further comprising the step, [prior to] following the activating step, of activating [the areas of conductivity] two conductive portions to cause [an] electroporation [of] in the target tissue.

57. (Amended) A method for making a molecule electromanipulator comprising the steps of:

affixing at least one [member containing areas of discrete conductivity] electrode member comprising conductive portions to a support in spaced-apart relation [each area of conductivity being differentially activatable];

providing circuit communication between each [conductivity area] conductive portion and a source of electrical energy, the conductive [areas] portions configured to establish a [low-level] first electromagnetic field *in vivo* between selected [conductivity areas] conductive portions for manipulating a molecule relative to a target tissue and a [higher-level] second

electromagnetic field *in vivo* for causing transient permeability of a cell membrane within the target tissue[;and

providing switching means between each conductivity area and the electrical energy source to permit differential activation of the areas of differing conductivity on each electrode member].

58. (Amended) The method recited in Claim 57, further comprising the step of providing means for controlling the switching means adapted to activate the [areas of conductivity] conductive portions in a preselected pattern.

59. (New) The device recited in claim 7, wherein the disc has a noncircular shape.

60. (New) The device recited in Claim 7, wherein the post has a geometry that facilitates grasping the device for accessing the target tissue.

61. (New) The device recited in Claim 1, further comprising at least two generally rectangular, striplike electrode members, each striplike electrode member movable between a first position and a second position, wherein the electrode members are closer together than in the first position.

62. (New) The device recited in Claim 60, further comprising a first restraining means for selecting the minimum distance between electrode members, and a second restraining means for selecting the maximum distance between electrode members.

63. (New) The device recited in Claim 61, wherein the first restraining means comprises an insert positionable in the lumen between the electrode members.

64. (New) The device recited in Claim 61, wherein the first restraining means comprises a set screw.

65. (New) The device recited in Claim 61, wherein the second restraining means comprises a torodial ring.

66. (New) The device recited in Claim 60, further comprising a lead in circuit communication with each conductive portion adapted for electrical communication with the source of electrical energy.

67. (New) The method recited in Claim 43, wherein the penetration is effected through biological tissue other than skin.

68. (New) The method recited in Claim 38, wherein the establishment of the second electrical potential causes the molecule to be delivered to the cytosol of the cells that comprise the target tissue.

69. (New) The method recited in Claim 38, wherein the first potential causing electromigration is used independently of electroporation.

70. (New) The method recited in Claim 38, wherein the second potential causing electroporation is used independently of electromigration.

71. (New) The method recited in Claim 38, wherein the electroporation is caused prior to the electromigration.

72. (New) The method recited in Claim 45, wherein the penetration is effected through biological tissue other than the skin.

73. (New) The method recited in Claim 44, wherein the electroporation of the second electrical potential causes the molecule to be delivered to the cytosol of the cells that comprise the target tissue.

74. (New) The method recited in Claim 44, wherein electromigration is used independently of electroporation.

75. (New) The method recited in Claim 44, wherein electroporation is used independently of electromigration.

76. (New) The method recited in Claim 44, wherein the electroporation is caused prior to the electromigration.

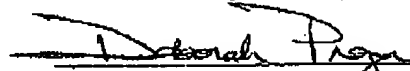
77. (New) The method recited in Claim 46, wherein the electromigration of the second molecule is effected to cause the second molecule to penetrate a skin layer.

78. (New) The method recited in Claim 57, further comprising switching means between each conductive portion and the electrical energy source to permit activation of the conductive portions on each electrode member.

CERTIFICATE OF FACSIMILE TRANSMISSION
(37 C.F.R. 1.8(a))

I HEREBY CERTIFY that this Exhibit A is being transmitted by facsimile to the United States Patent and Trademark Office, Art Unit 3763, Attn: Michael J. Hayes, (703) 872-9302.

Dated: March 26, 2002


Deborah Preza